Strain	Treatment	Elongation Rate (bp/s) <sup>a</sup>	ppGpp/GTP <sup>b</sup>	pppGpp/GTP <sup>b</sup>	ppGpp/ATP <sup>b</sup>	pppGpp/ATP <sup>b</sup>	GTP/ATP <sup>b</sup>
dnaC2	Untreated	651±23	$0.003 \pm 0.03$	$0.0002 \pm 0.03$	$0.016 \pm 0.01$	$0.008 \pm 0.02$	$0.602 \pm 0.03$
	SHX	565±27	2.29±0.23	$0.72 \pm 0.17$	$0.61 \pm 0.08$	$0.20 \pm 0.04$	$0.29 \pm 0.03$
$dnaC2 \Delta gppA$	Untreated	621±14	$0.05 \pm 0.02$	$0.05 \pm 0.02$	$0.02 \pm 0.01$	$0.03 \pm 0.01$	$0.55 \pm 0.05$
	SHX	403±21	2.41±0.21	2.43±0.24	$0.55 \pm 0.05$	$0.57 \pm 0.09$	$0.24 \pm 0.04$
dnaC2 pRelA*	Untreated	592±12	$0.15 \pm 0.03$	$0.18 \pm 0.04$	$0.08 \pm 0.02$	$0.07 \pm 0.06$	$0.51 \pm 0.03$
	IPTG	503±24	1.19±0.03	$0.51 \pm 0.13$	$0.55 \pm 0.06$	$0.14 \pm 0.05$	$0.46 \pm 0.02$
dnaC2 ΔgppA pRelA*	Untreated	604±17	$0.15 \pm 0.03$	$0.18 \pm 0.06$	$0.08 \pm 0.01$	$0.07 \pm 0.06$	$0.56 \pm 0.04$
	IPTG	386±12	1.33±0.06	1.16±0.03	0.59±0.02	$0.50 \pm 0.04$	$0.43 \pm 0.02$

<sup>&</sup>lt;sup>a</sup>±Range

Table S1. Replication elongation rates and nucleotide levels in E. coli following induction of (p)ppGpp.

Cells were treated with SHX (0.5 mg/ml) or treated with IPTG (1 mM) to induce expression of RelA\*. Replication rates were calculated by linear regression of a time course of the average replication fork positions generated by genomic microarrays. Nucleotide levels were determined by TLC and shown as molar ratios of the indicated nucleotide.

<sup>&</sup>lt;sup>b</sup>±SEM

Strain	Treatment	% Replication ( treated/t0)	ppGpp/GTP	pppGpp/GTP	ppGpp/ATP	pppGpp/ATP	GTP/ATP
$P_{hyperspank}$	Untreated	127±5	$0.004 \pm 0.08$	$0.01 \pm 0.007$	$0.002 \pm 0.02$	$0.01 \pm 0.002$	$0.63 \pm 0.03$
	Norv	36±2	$0.18 \pm 0.004$	$0.17 \pm 0.08$	$0.01 \pm 0.001$	$0.01 \pm 0.001$	$0.45 \pm 0.004$
	α-MG	20±1	$0.41 \pm 0.05$	$0.37 \pm 0.02$	$0.08 \pm 0.01$	$0.07 \pm 0.004$	$0.47 \pm 0.01$
	RHX	4±1	$0.48 \pm 0.03$	1.93±0.18	$0.07 \pm 0.0001$	0.27±0.006	0.11±0.01
P <sub>hyperspank</sub> -gppA	Untreated	106±12	$0.02 \pm 0.01$	$0.02 \pm 0.02$	$0.002 \pm 0.001$	$0.02 \pm 0.008$	$0.55 \pm 0.01$
	Norv	32±3	$0.45 \pm 0.12$	$0.62 \pm 0.07$	$0.02 \pm 0.001$	$0.02 \pm 0.005$	$0.38 \pm 0.01$
	α-MG	12±2	1.13±0.21	1.22±0.09	$0.03 \pm 0.001$	0.12±0.017	$0.12 \pm 0.03$
	RHX	6±1	1.26±0.21	1.55±0.31	0.18±0.02	0.16±0.014	0.10±0.01

Table S2. Relative replication rates and nucleotide levels in B. subtilis following induction of (p)ppGpp.

Cells were treated with 0.5 mg/ml norvaline (Norv), 2%  $\alpha$ -methyl-glucoside ( $\alpha$ -MG), and 0.5 mg/ml RHX. The relative rate of DNA replication is shown as the rate of incorporation of  $^3$ H-thymidine into DNA and expressed as a percentage of t0. Nucleotide levels were determined by TLC and shown as molar ratios of the indicated nucleotide. Error indicates SEM.

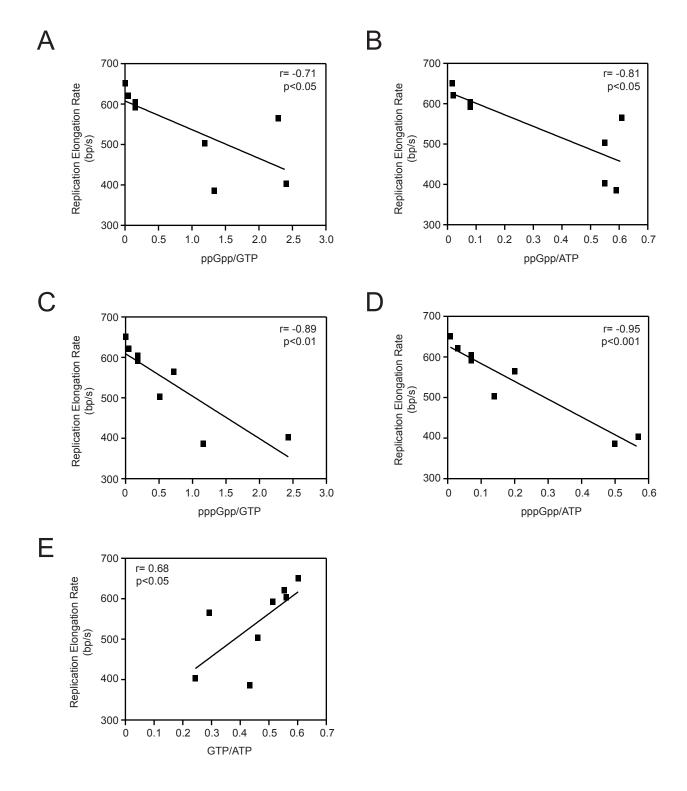


Figure S1. Replication elongation rates in *E. coli* relative to nucleotide levels under (p)ppGpp-inducing conditions.

Replication elongation rates (Table S1) were plotted against nucleotide levels presented as the molar ratio of the indicated nucleotide (Table S1). (A) ppGpp/GTP, (B) ppGpp/ATP, (C) pppGpp/GTP, (D) pppGpp/ATP, and (E) GTP/ATP. Data were fit by linear regression. r: Pearson's correlation coefficient.

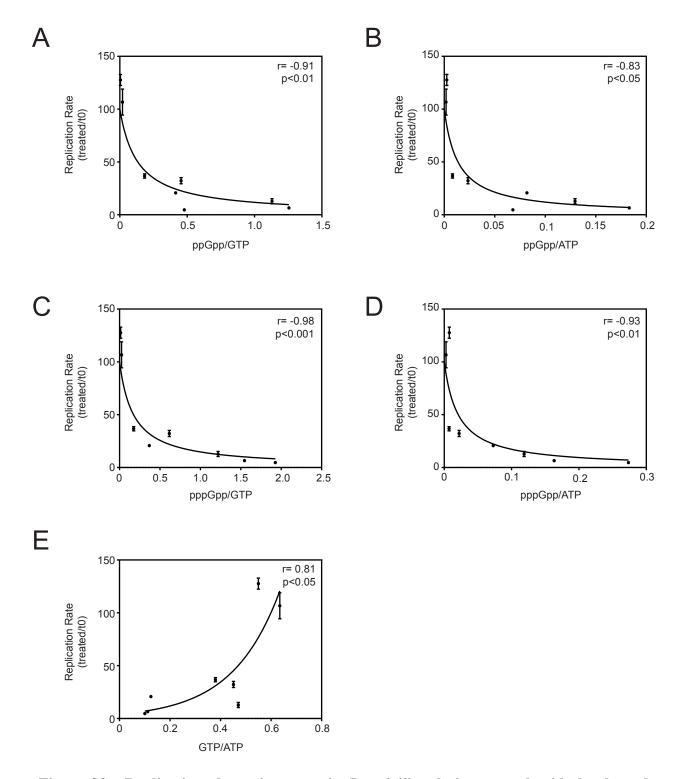


Figure S2. Replication elongation rates in *B. subtilis* relative to nucleotide levels under (p)ppGpp-inducing conditions.

Replication rates (Table S2) were plotted against nucleotide levels presented as the molar ratio of the indicated nucleotide (Table S2). (A) ppGpp/GTP, (B) ppGpp/ATP, (C) pppGpp/GTP, (D) pppGpp/ATP, and (E) GTP/ATP. (A-D) Data were fit to y=100/(1+(x/IC50)) where y is the replication rate, and x is the relative (p)ppGpp level. (E) Data were fit via exponential growth. r: Spearman's rank correlation coefficient.